Protein purification by Ni-NTA chromatography

Purify the filtered lysate with HisTrapTM (1 mL or 5 mL) with the following instructions:

Step	in	Column volumes (cv)	out
Pre-wash 1	dH_2O (Milli-	5	waste
Pre-wash 2	N_C buffer	5	waste
Loading	N_A buffer	5	waste
Lysate	all	all	flow
Wash 1	N_B	3	wash
wash 2	N_C buffer	until starts coming	wash 2
Elution	N_C buffer	until all has come	eluate
Cleaning elution	N_C buffer	2 – 3	waste
After-wash	dH_2O (Milli-	5	waste
storage	5 cv 20	5 – 10	waste

repeat procedure within dashed lines for a second protein sample

- Take SDS-samples from all collected fractions (flow through, wash) 50 µL + 20 µL 5xSDS-buffer
- From eluate take only 15 µL SDS-sample + 5 µL 5xSDS-buffer
- Dialyse protein in desired buffer (either storage buffer or TEV reaction buffer depending on following procedure)